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RELATION OF OXIDASES AND CATALASE TO RESPIRATION IN PLANTS

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The chemical mechanism of respiration in plants is very complex and imperfectly understood. It is a product of the living cells and is capable of bringing about, at low temperatures, the oxidation of organic substances which, in the laboratory, are oxidized only by employing very high temperatures and powerful reagents.

The presence of oxygen activators and carriers in plants has long been recognized and Traube as early as 1877 called these substances oxidizing ferments. Bertrand[6] was led to believe that the oxidizing ferments are more or less specific in their action, so he proposed the term oxidases as a group name for these ferments. Oppenheimer[11] has classed the oxidation enzymes according to the substances now generally recognized as acted upon by these enzymes. He realizes, however, the provisional character of such a classification and states further that the oxidation ferments represent the darkest Africa in the ferment world. The phenolases of Oppenheimer's classification accelerate only the oxidation of aromatic substances and are thus the oxidizing enzymes that are measured by the oxidase reagents in common use at the present time. The oxidases referred to in this paper include only the phenolases of Oppenheimer's classification.

Practically all attempts to explain the mechanism of respiration have assigned these long-established oxidative forces in the cell a rôle in this process, although such a relationship is almost entirely hypothetical. The chief difficulty lies in the fact that the oxidases, as detected and measured by the prevailing methods, have no direct action on the ordinary substances consumed in respiration, as sugar, for example. When it was recognized that respiration occurs in two distinct stages: namely, an anaerobic and aerobic stage, the oxidases were relieved of the responsibility of direct oxidation of these complex organic substances; since on this basis they would be concerned only in the oxidation of the decomposition products of the anaerobic stage. But all the known products of this stage are aliphatic substances incapable of being directly oxidized *in vitro* by the oxidases; therefore, the situation remains nearly as perplexing as ever.

The very ingenious hypothesis elaborated by Palladin[12] and his co-workers to explain the mechanism of respiration comes the nearest of anything yet offered to overcome the chief difficulties encountered in ascribing to the oxidases a function in respiration. It must be remembered, however, that this explanation is still in the hypothetical stage.

There are certain substances in probably all plant and animal cells which have the power to decompose hydrogen peroxide with the evolution of molecular oxygen. Loew[9] thought that this action on hydrogen peroxide is due to a special enzyme to which he gave the name catalase. When one considers the abundance and wide distribution of catalase in plant and animal tissues, it is natural to suppose that it plays some important rôle in metabolism. But so far its function has not been definitely established. Much theory, based upon rather scanty experimental data, has attempted directly or indirectly to connect catalase activity with the oxidative forces of the cell. The work of Lesser[10] seems to furnish the most conclusive evidence in this direction. He made a large number of catalase determinations in different small animals and in different organs and tissues of the same animal and concluded that catalase is connected with physiological oxidations. Although a strict interpretation of his results does not show a casual relation between catalase and cell oxidation, it does show a remarkable correlation. Zieger[13] also made a study of the catalase content of nearly all groups of animals except the protozoa. He did not succeed in establishing a relationship between intensity of respiration and catalase activity, but he did show that there is some relation between catalase content and metabolism. He brought out this fact by the study of the catalase content in organs which are very active chemically, as the liver and kidney. The evidence from the plant side, for such a relationship, is indirect and inconclusive. Kohl[8] indirectly connects catalase activity with respiration by his claim that it functions in alcoholic fermentation.

In conformity with the literature both the oxidases and catalase have been spoken of as enzymes although in the light of our present knowledge their place in the category of enzymes is extremely doubtful. It is questionable whether they are even definite chemical substances. It may be more correct to speak of oxidase and catalase activity rather than oxidases and catalase. This paper, however, is not concerned with the chemical nature or mode of action of these substances, nor is it concerned with any particular theory of respiration. Therefore, an exhaustive citation of the literature is not pertinent to the matter in hand. The sole object in view was a quantitative study of the relation of both oxidase and catalase activity to intensity of respiration. Potato tubers seemed especially favorable material for a study of this kind, since respiration in these tubers is greatly accelerated by various artificial treatments and is subject to fluctations under natural conditions, as greening, sprouting, etc. The rate of respiration also varies in different parts of the same tuber and tubers of different varieties. Besides, these tubers contain very active oxidases and catalase. The modification of the intensity of respiration in the tubers was determined and at the same time measurements were made of both the oxidase and catalase activity in the juice.

METHODS

Respiration: The rate of respiration was determined by the amount of CO_2 expired from the tubers. No attempt was made to control the temperature, but all measurements that are compared were made at the same time and under identical conditions. Tubers of about a kilo's weight were allowed to respire twenty-four hours for each determination.

Oxidase: A manometric method was used for the oxidase determinations, the oxygen absorbed during the reaction being measured by the change of pressure within Bunzel's simplified apparatus. Both pyrocatechin and hydrochinon were first employed as the oxidizable substance, but it was soon found that they showed the same general relations in respect to oxidase activity under different conditions. Since the reaction with hydrochinon was very slow as compared with that of pyrocatechin, its use was soon abandoned in favor of pyrocatechin as the sole reagent. From Bunzel's[4] work on the "Oxidases in Healthy and in Curly-Dwarf Potatoes" in which he used 18 ring compounds, it may be concluded that the use of one favorable compound of this nature would give just as valuable comparative results as use of a larger number. He found a wide variation in the amount of oxygen absorbed by the different compounds, but aside from one or two exceptions, they all showed the same general relation of oxidase activity in the different juices examined. In another paper, Bunzel[5] states: "No matter what the derivation of the plant juice is, the relative oxidizability of the various compounds is approximately the same."

Bunzel's[3] apparatus, of the size now on the market, required very small amounts of juice for a determination of the oxidase in the tubers of the principal variety employed in this work. In order to avoid errors in the measurement of extremely small quantities of juice, resort was made to the following dilution method: 2 cc. of juice were measured into 10 cc. of distilled water and after thorough mixing, without violent shaking, 2 cc. of the dilution were placed in the long arm and 5 milligrams of oxidizable substance in the short arm of the apparatus. The determinations were made in a constant temperature box of 33° C. During the reaction the apparatus was shaken constantly at the rate of 180 complete excursions per minute. After repeated trials with the diluted juice, it was found that the rate of shaking within wide limits had no effect on the total amount of oxygen absorbed; but the velocity of the reaction seemed to be greatest at about the amount of shaking decided upon for the standard in this work.*

The manometer readings recorded in this paper are those made at the end of one hour, unless otherwise noted, and represent the oxidase activity in .33 cc. of undiluted juice. Although in the case of pyrocatechin a very slow oxidation continued for several hours, it came to a comparatively definite end point after an hour.

Catalase: The potato juice for the catalase determinations was prepared by grating the tubers with calcium carbonate in the manner described by the writer[1] in a previous paper. The calcium carbonate neutralizes the free acids which very rapidly destroy the catalase in the juice. The catalase measurements were made in the same kind of apparatus used for the oxidase measurements, except that the apparatus was graduated to read positive pressures. The juice for a catalase determination was diluted in the following manner: 2 cc. of juice were added to 25 cc. of distilled water. The juice was thoroughly mixed with the water by rotating the flask 25 times. One cubic centimeter of the mixture was carefully measured into the long arm of the apparatus and 1 cc. of Oakland dioxogen (hydrogen peroxid) in the short arm. The determinations were made in the same constant temperature box and shaken with the same shaker used for oxidase. The manometer reading at the end of five minutes constant shaking was considered the measure of catalase activity in .074 cc. of juice. All the catalase results, in the experimental part, represent the activity in this amount of juice, except when otherwise noted. The oxidase and catalase measurements were all made in duplicate and those whose results were not in close agreement were discarded.

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* The shaking machine is described in bulletin No. 191 from the Maryland Agricultural Experiment Station.

It was very desirable to use the same lot of juice for both catalase and oxidase measurements, but before this was possible it was necessary to determine what effect the $CaCO_3$ would have upon the oxidase activity. To this end, tubers were cut in half longitudinally so that each piece contained exactly one half of the seed and stem ends. One piece was grated without $CaCO_3$ and the other by dipping into $CaCO_3$ in the usual way for catalase determinations. Oxidase measurements were then made in the juice thus prepared. The average of seven determinations was exactly the same in both cases. Therefore, it is quite evident that the $CaCO_3$ exercises no appreciable effect upon the oxidase activity in potato juice, according to the method here employed for its measurement.

The above conclusions regarding the effect on oxidase activity of $CaCO_3$ in the juice apply only with pyrocatechin. The effect may be otherwise with other chromogens. In fact, the presence of $CaCO_3$ in the juice seems to depress the oxidation of aloin, while the peroxidase activity is actually stimulated both in the case of aloin and guaiaconic acid.

EXPERIMENTAL RESULTS

Ethyl Bromide: In a previous paper the writer[2] has shown that short exposures to ethyl bromide gas will about double the respiration in old McCormick potatoes. This treatment was repeated and catalase and oxidase determinations made on the same tubers used for respiration. Duplicate determinations on untreated tubers were made at the same time and under the same conditions. The results show that the treatment has no effect whatever on the oxidases. The catalase activity on the other hand is greatly increased in the treated tubers.

TABLE I

Respiration of McCormick Potatoes After an Exposure of the Tubers to Ethyl Bromide Gas for Thirty Minutes

Date of Measurement	Time Elapsed After Treatment with Ethyl Bromide Gas	Milligrams of CO_2 per Kilo per Hour		Ratio
		Untreated	Ethyl Bromide Gas—30 Min.	
February 12	1 hour	19.17	37.94	1 : 1.98
February 24	12 days	16.60	18.54	1 : 1.27

TABLE II

Oxidase Activity in Juice from McCormick Potatoes After an Exposure of the Tubers to Ethyl Bromide Gas for Thirty Minutes

Experiment	Time Elapsed After Treatment with Ethyl Bromide Gas, Hours	Quantity of Juice Used, Cc.	Manometer Readings Expressed in Centimeters of Mercury	
			Untreated	Ethyl Bromide Gas—30 Min.
1	1	1	− 4.4	− 4.5
2	1	1	− 4.5	− 4.5
3	2	.5	− 3.0	− 3.2
4	24	.5	− 3.2	− 3.4
5	24	.2	− 1.0	− 1.0
6	48	.33	− 2.5	− 2.5
Average........	....	....	− 3.1	− 3.18

TABLE III

Catalase Activity in Juice from McCormick Potatoes After an Exposure of the Tubers to Ethyl Bromide Gas

Experiment	Time Elapsed After Treatment with Ethyl Bromide Gas—Hours	Manometer Readings Expressed in Centimeters of Mercury	
		Untreated	Ethyl Bromide Gas—30 Min.
1	20	+ 2.6	+ 3.5
2	21	+ 2.2	+ 3.6
3	40	+ 2.0	+ 3.5
4	40	+ 2.4	+ 3.5
Average	....	+ 2.3	+ 3.53

Cold Storage: If tubers are stored for a few weeks at low temperatures and then brought to room temperature, they respire much more rapidly than tubers of the same lot which were not subjected to the cold storage. The effect on the catalase and oxidase is almost identical with that under the ethyl bromide treatment, if the cold storage temperature does not fall below 3° C. In a previous work, the writer found that at temperatures around 0° C., or below, the catalase activity is actually less than in normally stored tubers. This was accounted for in the destruction of the catalase by free acids. The presence of free acids is demonstrated by the acid exudate from tubers stored at this very low temperature.

TABLE IV

Effect of Cold Storage on Respiration of McCormick Potatoes

Date of Measurement	Milligrams of CO_2 per Kilo per Hour		Ratio
	Tubers Stored at Room Temperature	Tubers Stored at 3° for 20 Days	
February 18	12.50	35.15	1 : 2.81

TABLE V

Catalase and Oxidase Activity in the Juice from McCormick Tubers after a Period of Cold Storage

Experiment	Manometer Readings Expressed in Centimeters of Mercury, Using .1 cc. of Juice for Catalase and .5 cc. for Oxidase			
	Tubers Stored at Room Temperature		Tubers Stored at 3° C. for 20 Days	
	Catalase	Oxidase	Catalase	Oxidase
1	+ 2.4	− 3.0	+ 4.2	− 2.9
2	+ 3.5	− 2.9	+ 4.5	− 2.7
3	+ 3.6	− 2.9	+ 4.2	− 2.3
4	+ 2.4	.....	+ 4.4	.....
Average	+ 2.97	− 2.93	+ 4.32	− 2.63

Effect of Greening: Greening of potato tubers in light is a very familiar phenomenon. One of the physiological changes concomitant with the greening is a rise in respiration. This offered a good opportunity to make a quantitative study of catalase and oxidase in relation to a change in respiration naturally induced. In connection with other work, the writer found in different stages of greening, a much greater rise in respiration than in the experiments here recorded. Even with the degree of acceleration shown in Table VI there is an increase in catalase in the green tubers. But again, the oxidase activity is not increased with a rise in respiration; in fact, it is a little less in the green tubers than in the ungreened ones.

TABLE VI

Effect of Greening on Respiration, Catalase and Oxidase

Experiment	Milligrams of CO_2 per Kilo per Hour		Manometer Readings Expressed in Centimeters of Mercury			
			Catalase		Oxidase	
	Normal	Green	Normal	Green	Normal	Green
1	17.14	19.50	+ 2.5	+ 2.8	− 2.35	− 2.1
2	14.32	16.24	+ 2.1	+ 2.5	− 2.2	− 2.1

Effect of Sprouting: Whole tubers of many varieties produce sprouts only from the buds on the seed end. Tubers of such varieties were cut in half and respiration measured separately in the seed and stem halves. It was found that respiration is always much higher in the seed halves when the sprouts are left on. This difference seems to be greater during incipient sprouting than after the sprouts have attained considerable size. How much of the increased respiration in the seed ends bearing the sprouts is due to the respiration of the sprouts themselves is difficult to determine. If the sprouts are removed just prior to the measurement of respiration, the results are quite different and depend upon the variety in question. In all cases the difference in respiration between the seed and stem ends is suddenly reduced. In McCormick tubers, it always remains a little higher in the seed halves.

The behavior of the catalase activity in the two ends of the McCormick tuber is practically identical with that of respiration. No difference in the oxidase activity of the two ends could be detected by the Bunzel method, using either pyrocatechin or pyrogallol as the oxidizable substance.

TABLE VII

Comparison of Respiration in Seed and Stem Ends; McCormick Tubers, Sprouting Only From Seed Ends

Sprouts	CO_2 per Kilo per Hour Expressed in Ratio of Seed and Stem Ends	
	Seed Ends	Stem Ends
Not started	100	93.5
Not started	100	93.2
On during measurement	100	58.2
Removed prior to measurement	100	80.2
Removed prior to measurement	100	89.3

The results of a large number of colorimetric determinations, using aloin as the oxidizable substance, show that this method does not agree with the Bunzel method in indicating the relative oxidase activity in the two ends of McCormick tubers. The colorimetric method consistently showed a greater oxidase activity in the stem half of the tuber, although this half always exhibited a lower rate of respiration. Therefore, neither method disclosed any relation between oxidase activity and the intensity of respiration.

TABLE VIII

Catalase and Oxidase Activity in Seed and Stem Ends, McCormick Tubers Sprouting Only From Seed Ends

Date of Measurement	Average Length of Sprouts	Manometer Readings Expressed in Centimeters of Mercury			
		Seed Ends		Stem Ends	
		Catalase	Oxidase	Catalase	Oxidase
March 20	0	+ 2.4	− 1.7	+ 2.1	− 1.9
March 22	0	+ 3.1	− 2.1	+ 2.7	− 2.2
March 25	8 mm.	+ 4.0	− 2.2	+ 3.1	− 2.2
April 6	12 mm.	+ 3.6	− 2.25	+ 3.0	− 2.1
Average		+ 3.36	− 2.06	+ 2.72	− 2.12

Comparison of Different Varieties: Tubers from different varieties, but under identical storage and sprouting conditions, were found to possess different rates of respiration. This fact being established, experiments were next planned to determine if there is a corresponding

TABLE IX

Respiration of Tubers from Two Different Varieties. All Conditions of Experiment Identical in Both Cases

Date of Measurement	Milligrams of CO_2 per Kilo per Hour	
	McCormick	Carman No. 1
April 21	12.83	17.36

TABLE X

Catalase and Oxidase Activity in Juice from Tubers of Two Different Varieties

Date of Measurement	Manometer Readings Expressed in Centimeters of Mercury			
	Catalase		Oxidase	
	McCormick	Carman No. 1	McCormick	Carman No. 1
April 14	2.3	3.8	2.2	.60
April 23	2.85	3.15	2.6	.64

difference in either the catalase or oxidase activity in the varieties showing a difference in rate of respiration. A varietal influence on both catalase and oxidase was soon discovered, although it is much greater in some cases than in others. The most striking difference was noted in the case of McCormick and Carman No. 1. Both varieties were under identical storage conditions for nearly two months prior to the date of experiment and both bore sprouts of practically the same vigor as determined by length and total weight. The sprouts were removed just before the measurement of the rate of respiration. The Carman No. 1 tubers respired more rapidly than the McCormick tubers. The catalase activity in the two varieties was strikingly correlated with respiration. On the other hand, the oxidase activity was four times greater in the McCormick tubers. Tables IX and X show typical experiments with these two varieties.

Summary and Conclusions

The introduction contains a statement of the fundamental difference between physiological oxidation as it occurs in respiration and ordinary combustion of organic substances in air. This is followed by a brief discussion of oxygen activators and carriers in plants and the difficulties encountered in assigning to the oxidases a rôle in respiration.

The important literature pertaining to catalase in its relation to respiration is reviewed.

The task was a quantitative study of the relation of both oxidase and catalase activity to intensity of respiration. Potato tubers seemed especially favorable material for a study of this kind since respiration in these tubers is greatly accelerated by various artificial treatments and is subject to fluctuation under natural conditions, as greening, sprouting, etc. The rate of respiration also varies in different parts of the same tuber and in tubers of different varieties. Besides, potato tubers contain very active oxidases and catalase. The modification of the intensity of respiration in these tubers was determined and at the same time measurements were made of both the oxidase and catalase activity in the juice. The data seem to justify the following conclusions:

1. The oxidase content in potato juice gives no indication of the intensity of respiration in the tubers. In other words, there is no

correlation between oxidase activity and the rate of respiration in these organs. The author does not disclaim any rôle of the demonstrable oxidases in respiration, but they certainly are not the controlling factor in regulating the rate of respiration in potato tubers.

2. Catalase activity in the potato juice shows a very striking correlation with respiratory activity in the tubers.

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